

WHAT IS CLAIMED IS:

1                   1.       A method of making a non-replicating anti-bacterial phage, said  
2 method comprising the step of producing said anti-bacterial phage in a host production  
3 bacterium, wherein said anti-bacterial phage is unable to replicate in a target bacterium and  
4 wherein said anti-bacterial phage inhibits growth of said target bacterium.

1                   2.       The method of Claim 1, wherein said non-replicating anti-bacterial  
2 phage is unable to replicate in said target bacterium because:  
3                               the nucleic acid of said anti-bacterial phage is inactivated or removed;  
4                               said phage comprises a mutation and cannot assemble into a replication  
5 competent phage in said target bacterium, but said host production bacterium is a  
6 complementing host bacterium that is able to complement, including with a helper phage,  
7 said mutation of said anti-bacterial phage and allow replication of said anti-bacterial phage in  
8 said complementing host production bacterium;  
9                               said phage comprises DNA containing a restriction site sensitive to a  
10 restriction enzyme activity, said activity found in said target bacterium but absent in said host  
11 production bacterium; or  
12                               said phage expresses in said target bacterium a function early in  
13 infection which prevents DNA or phage replication, but fails to express said function in said  
14 host production bacterium.

1                   3.       The method of Claim 2, wherein:  
2                               said mutation is temperature sensitive at a non-permissive temperature,  
3 and said complementing host production bacterium complements said mutation at said non-  
4 permissive temperature;  
5                               a nucleic acid of said non-replicating anti-bacterial phage comprises a  
6 mutation and cannot assemble into a replication competent phage, further comprising a step  
7 of supplying a complementing helper phage that can complement said mutation of said anti-  
8 bacterial phage and allow replication of said anti-bacterial phage in said host production  
9 bacterium;  
10                               said mutation is a substantial deletion, and said complementing host  
11 production bacterium complements said deletion mutation, e.g., with a gene in said host  
12 production bacterium or a helper phage;

13                               said host production bacterium expresses an inhibitor of expression or  
14 function of said restriction enzyme;  
15                               said function early in infection is a nuclease which prevents DNA or  
16 phage replication; or  
17                               said function early in infection is blocked in said host production  
18 bacterium by antisense message expression.

1                           4.     A pharmaceutically acceptable complementing host production  
2 bacterium used in a method of Claim 1.

1                           5.     A pharmaceutical composition comprising an anti-bacterial phage,  
2 wherein said anti-bacterial phage inhibits growth of a target bacterium, and wherein said anti-  
3 bacterial phage has diminished replication activity in said target bacterium.

1                           6.     The composition of Claim 5, wherein:  
2                               said anti-bacterial phage exhibits no DNA or phage replication activity  
3 in said target bacterium;  
4                               said anti-bacterial phage comprises less than 98% of the complexity of  
5 the nucleic acid of an intact phage;  
6                               said anti-bacterial phage comprises less than 20% of the nucleic acid  
7 content of an intact parental phage;  
8                               said anti-bacterial phage comprises less than 2% of the nucleic acid  
9 content of the intact parental phage;  
10                              said anti-bacterial phage does not contain detectable nucleic acid;  
11                              said anti-bacterial phage comprises an intact phage comprising nucleic  
12 acid with a reduced replication capacity in said target bacterium;  
13                              said anti-bacterial phage comprises a tail portion of a tailed phage,  
14 including a myoviridae or syphoviridae phage;  
15                              said anti-bacterial phage comprises an electron microscope  
16 morphologically identifiable tail portion of a tailed phage;  
17                              said anti-bacterial phage consists essentially of a tail portion of a  
18 myoviridae or syphoviridae phage;  
19                              said composition further comprises a therapeutically compatible buffer  
20 or excipient;

21 said composition further comprises a second therapeutic agent,  
22 including an anti-microbial, antibiotic, or inflammatory agent;

23 said anti-bacterial phage is made by a method comprising the steps of:  
24 a) amplifying a phage in a host production bacterium,  
25 b) harvesting said phage from said host production bacterial  
26 culture, and  
27 c) depleting or inactivating substantially all of the nucleic acids  
28 from said phage, thereby producing said anti-bacterial phage;

29 said anti-bacterial phage is made by a method comprising steps of:  
30 a) amplifying a phage in a host production bacterium, and  
31 b) harvesting said phage from said host production bacterial  
32 culture before substantial amounts of intact phage are produced or assembled, thereby  
33 producing said anti-bacterial phage; or

34 said anti-bacterial phage is made by a method comprising steps of:  
35 a) amplifying a phage in a host production bacterium, and  
36 b) harvesting said phage from said host production bacterial  
37 culture, wherein a nucleic acid of said anti-bacterial phage comprises a mutation and  
38 cannot assemble into a replication competent phage, and wherein said host production  
39 bacterium is a complementing host production bacterium that is able to complement  
40 said mutation of said anti-bacterial phage and allow replication of said anti-bacterial  
41 phage in said complementing host production bacterium, including where said  
42 complementing results from a helper phage, thereby producing said anti-bacterial  
43 phage.

1 7. A method of treating a bacterial population:  
2 in a subject in need of said treatment, said method comprising  
3 administering a therapeutically effective amount of a composition of Claim 5; or  
4 in a subject, said method comprising administering a prophylactically  
5 effective amount of a composition of Claim 5.

1 8. The method of Claim 7, wherein:  
2 said bacterial infection is caused by said target bacterium;  
3 said subject is a human;  
4 said subject is a primate, a food, work, display, or a companion animal;

5 said target bacterium is Escherichia, Staphylococcus, Pseudomonas, or  
6 Streptococcus;

7 said method further comprises administering a second therapeutic or  
8 antimicrobial agent, including administering systemically, parenterally, orally, topically, or  
9 by inhalation, catheter, or drain tube;

10 said method results in a relative decrease in said population of at least  
11 10-1000 fold; or

12 said method results in a decrease in detectability of said population by  
13 at least 5-50 fold.

1 9. A pharmaceutical composition comprising a genetically incompetent  
2 anti-bacterial phage, wherein said anti-bacterial phage inhibits growth of a target bacterium.

1 10. The pharmaceutical composition of Claim 9, wherein:

2 said target bacterium is identified or diagnosed, including an  
3 Escherichia, Staphylococcus, Pseudomonas, or Streptococcus bacterium;

4 said genetically incompetent anti-bacterial phage lacks a full  
5 complement of genetic material;

6 said genetically incompetent anti-bacterial phage has a mutation and  
7 cannot assemble into replication competent phage in said target bacterium;

8 said genetically incompetent anti-bacterial phage comprises nucleic  
9 acid with a reduced replication capacity, e.g., comprising a mutation, including a missense,  
10 termination, frameshift, conditional, deletion, or insertion mutation, in a critical phage  
11 replication function;

12 said genetically incompetent anti-bacterial phage consists essentially of  
13 a tail portion from a tailed phage, including a myoviridae or syphoviridae phage; or

14 said pharmaceutical composition further comprises an excipient,  
15 buffer, or a second therapeutic or anti-microbial agent.

1 11. A method of using a pharmaceutical composition of Claim 9 to treat a  
2 bacterial infection in a subject in need of such treatment, said method comprising a step of  
3 administering a therapeutically effective amount of said pharmaceutical composition.

1 12. The method of Claim 11, wherein:

2 said subject is a human;

3                   said subject is a primate, a food, work, display, or companion animal;  
4                   said pharmaceutical composition is administered systemically,  
5 parenterally, orally, topically, or by inhalation, catheter, or drain tube; or  
6                   said pharmaceutical composition is administered in combination with a  
7 second therapeutic or anti-bacterial agent, e.g., an anti-microbial, inflammatory, or anti-  
8 inflammatory agent.

1                   13.    A method of identifying an anti-bacterial phage that is unable to  
2 replicate in a selected target bacterium, said method comprising the steps of:  
3                   culturing said target bacterium; and  
4                   testing various potential anti-bacterial phage, including genetic variants  
5 of a phage, for combined properties of inhibition of growth on said target bacterium, and  
6 absence of capacity to replicate phage DNA or phage in said target bacterium.

1                   14.    An anti-bacterial phage that is identified using said method of Claim  
2 13, wherein said phage inhibits growth of a target bacterium and is unable to replicate in said  
3 target bacterium. [product by process claim, but might be difficult to enforce]

1                   15.    A method of producing non-replicating anti-bacterial phage  
2 comprising the steps of:  
3                   replicating phage in a host production bacterium,  
4                   harvesting said phage from said host production bacterial culture, and  
5                   removing substantially all of the function of the nucleic acids from said  
6 phage, thereby producing said non-replicating anti-bacterial phage.

1                   16.    The method of Claim 15, wherein:  
2                   said anti-bacterial phage is a tailed phage, including a myoviridae or  
3 syphoviridae phage;  
4                   said nucleic acids are removed by steps of:  
5                   a)    separating tails from heads of tailed phage fragments, and  
6                   b)    isolating said tails;  
7                   said function of said nucleic acids is removed by steps of:  
8                   a)    harvesting said phage before tails and heads have assembled to  
9 form an intact phage, and  
10                  b)    isolating said tails;

11                               said function of said nucleic acids is removed by osmotic shock, a  
12 freeze-thaw cycle, a chemical method, or a mechanical method; or  
13                               said function of said nucleic acids is removed by genetic mutation,  
14 e.g., a missense, termination, frameshift, conditional, deletion, or insertion mutation.

1                               17.     A method of making a defined dose anti-bacterial phage that kills a  
2 defined target bacterium, said method comprising producing said anti-bacterial phage in:  
3                               a host production bacterium and isolating tail portions separate from  
4 DNA containing heads;  
5                               a host production bacterium and inactivating nucleic acid of said  
6 phage, e.g., by nicking, fragmenting, crosslinking, or chemically modifying said nucleic acid;  
7                               a host production bacterium and harvesting components temporally  
8 before substantial assembly of complete phage;  
9                               a complementing host production bacterium where said anti-bacterial  
10 phage would not replicate in said target bacterium;  
11                               a host production bacterium comprising a helper phage where said  
12 anti-bacterial phage would not replicate in said target bacterium; or  
13                               a permissive production host which phage are non-permissive for  
14 replication in target bacterium in a different condition, e.g., temperature.

1                               18.     The method of Claim 17, wherein:  
2                               said anti-bacterial phage is a tailed phage, including a myoviridae or  
3 syphoviridae tailed phage;  
4                               said anti-bacterial phage is produced in a complementing host  
5 production bacterium or with a complementing helper phage, wherein the coding nucleic acid  
6 for said anti-bacterial phage comprises, in a critical gene necessary for phage replication in  
7 said target bacterium, a mutation, e.g., a missense, termination, frameshift, conditional,  
8 deletion, or insertion;  
9                               said anti-bacterial phage exhibits less than 5% of the DNA or phage  
10 replication activity in said target bacterium compared to that exhibited by intact phage in said  
11 host production bacterium;  
12                               said anti-bacterial phage exhibits diminished capacity to transmit toxin  
13 genes in said target bacteria when compared to intact phage in said host bacterium;

14                   said anti-bacterial phage exhibits diminished immunogenicity  
15 compared to intact phage from said host bacteria upon administration to a mammal, e.g., by  
16 30%, 60%, 90%, 95%, or 99%, in immune response or number of epitopes over a period of  
17 treatment exposure;  
18                   said anti-bacterial phage exhibits no significant DNA replication or  
19 phage replication activity in said target bacterium;  
20                   said target bacterium is a pathogenic bacterium, including a  
21 nosocomial or pyogenic bacterium, a Gram negative bacterium, or an Escherichia,  
22 Staphylococcus, Pseudomonas, or Streptococcus bacterium;  
23                   said target bacterium is a food or environmental contaminant; or  
24                   a second technique is used to inactivate or remove remaining DNA in  
25 said defined dose anti-bacterial phage.

1                   19.   The complementing host or helper phage of Claim 18B, wherein said  
2 host production bacterium or helper phage encodes one or more genes which complement  
3 said mutation in said anti-bacterial phage, thereby allowing said anti-bacterial phage to  
4 replicate in said producing bacterium.

1                   20.   A defined dose therapeutic anti-bacterial composition comprising a  
2 phage protein derived from an intact parental phage or prophage, said anti-bacterial  
3 composition capable of killing a target bacterium, said anti-bacterial composition exhibiting  
4 less than 20% DNA or phage replication activity in said target bacterium, when compared to  
5 said intact parental phage or prophage.

1                   21.   The composition of Claim 20, wherein:  
2                   said composition exhibits less than 5% replication activity in said  
3 target bacterium when compared to said intact parental phage;  
4                   said anti-bacterial phage exhibits diminished capacity to transmit toxin  
5 genes in said target bacteria when compared to intact phage in said host bacterium;  
6                   said anti-bacterial composition exhibits diminished immunogenicity  
7 compared to said intact phage from a host bacteria upon administration to a mammal;  
8                   said anti-bacterial phage exhibits no substantial or detectable DNA or  
9 phage replication activity in said target bacterium;

10                   said target bacterium is a pathogenic bacterium, including a  
11   nosocomial or pyogenic bacterium, or a Gram negative bacterium, such as Escherichia,  
12   Staphylococcus, Pseudomonas, or Streptococcus bacterium;  
13                   said target bacterium is a food or environmental contaminant;  
14                   said composition further comprises a nucleic acid with reduced  
15   replication capacity, e.g., where the nucleic acid has been nicked, fragmented, cross linked, or  
16   UV irradiated;  
17                   said composition comprises less than 20% of the nucleic acid content  
18   of said intact parental phage;  
19                   said composition lacks detectable nucleic acid;  
20                   said composition comprises a damaged DNA that is unable to be  
21   replicated;  
22                   said intact parental phage is a tailed phage, including a myoviridae or  
23   syphoviridae phage, and said composition comprises a tail portion or a tail protein;  
24                   said composition further comprises a therapeutically compatible buffer  
25   or excipient;  
26                   said composition further comprises a second therapeutic or anti-  
27   microbial agent, e.g., an antibiotic or a bacterial cell wall growth disrupting compound;  
28                   said anti-bacterial composition is made by a method comprising the  
29   step of processing said intact parental phage to remove or inactivate nucleic acids;  
30                   said anti-bacterial composition is made by a method comprising the  
31   step of harvesting phage from a host bacterium before intact phage are assembled from  
32   components thereof; or  
33                   said anti-bacterial composition is made by a method comprising the  
34   step of expressing in a complementing host production strain a phage genome defective in  
35   expressing a critical gene for replication, infection, assembly, production, or release by said  
36   phage, including where said phage genome comprises a mutation, including a missense,  
37   termination, frameshift, conditional, deletion, or insertion, which prevents phage replication  
38   in said target bacterium.

1                   22.   A method of treating a bacterial colonization in a eukaryote  
2   experiencing colonization by said target bacterium, said method comprising administering a  
3   composition of Claim 20 to said eukaryote.



1                   23.    The method of Claim 22, wherein:  
2                            said eukaryote is a mammal, including a primate;  
3                            said eukaryote is a food, work, display, or companion animal;  
4                            said target bacterium is a pathogenic, nosocomial, or pyogenic  
5 bacterium;  
6                            said target bacterium is an Escherichia, Staphylococcus, Pseudomonas,  
7 or Streptococcus bacterium;  
8                            said composition is administered systemically, parenterally, orally,  
9 topically, or by inhalation, catheter, or drain tube;  
10                          said colonization has already been treated with an anti-microbial or  
11 antibiotic;  
12                          said colonization has been diagnosed to be susceptible to the selected  
13 composition; or  
14                          said eukaryote is also inoculated with another bacterium to replace said  
15 target bacterium.

1                   24.    A therapeutic anti-bacterial composition comprising a genetically  
2 incompetent phage wherein said phage kills a target bacterium.

1                   25.    The composition of Claim 24, wherein:  
2                            said phage lacks detectable nucleic acid;  
3                            said phage comprises a chemically or physically damaged nucleic acid;  
4                            said phage lacks a functional gene necessary to replicate phage DNA  
5 in said target bacterium, or contains a gene which prevents replication of phage DNA in said  
6 target bacterium (restriction/modification or phage exclusion system);  
7                            said phage comprises a missense, termination, frameshift, conditional,  
8 deletion, or insertion mutation in a gene necessary for phage replication, e.g., capacity to  
9 infect, assemble, produce, or release intact phage, or contains a gene whose expression  
10 prevents phage replication (restriction/modification system);  
11                          said phage comprises a tail protein from a tailed phage;  
12                          said composition is used therapeutically to treat a food, work, display,  
13 or companion animal, or primate;  
14                          said target bacterium is a pathogenic bacterium, e.g., an Escherichia,  
15 Staphylococcus, Pseudomonas, or Streptococcus bacterium;

16                               said composition is administered systemically, parenterally, orally,  
17   topically, or by inhalation, catheter, or drain tube; or  
18                               said composition is administered in combination with a second  
19   therapeutic agent, including an anti-bacterial, inflammatory, or anti-inflammatory agent.